PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 2004367-0046	FOR FURTHER ACTION	See Form PCT/IPEA/416
International application No. PCT/US05/03092	International filing date *(day/month/year)* 27 January 2005 (27.01.2005)	Priority date *(day/month/year)* 27 January 2004 (27.01.2004)
International Patent Classification (IPC) or national classification and IPC IPC(7): A61F 2/28 and US Cl.: 424/426; 523/114,115		
Applicant OSTEOTECH, INC.		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 3 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☒ *(sent to the applicant and to the International Bureau)* a total of 7 sheets, as follows:
      ☐ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) _____ , containing a sequence listing and/or tables related thereto, in electronic form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).
4. This report contains indications relating to the following items:
   - ☒ Box No. I Basis of the report
   - ☐ Box No. II Priority
   - ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - ☐ Box No. IV Lack of unity of invention
   - ☒ Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - ☐ Box No. VI Certain documents cited
   - ☐ Box No. VII Certain defects in the international application
   - ☐ Box No. VIII Certain observations on the international application

Date of submission of the demand	Date of completion of this report
06 September 2005 (06.09.2005)	02 December 2005 (02.12.2005)
Name and mailing address of the IPEA/ US Mail Stop PCT, Attn: IPEA/US Commissioner for Patents P.O. Box 1450 Alexandria, Virginia 22313-1450 Facsimile No. (571) 273-0588	Authorized officer Carlos A. Azpuru Telephone No. 703-308-1235

Form PCT/IPEA/409 (cover sheet)(April 2005)

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/US05/03092

Box No. I Basis of the report

1. With regard to the **language**, this report is based on:

☒ the international application in the language in which it was filed.

☐ a translation of the international application into English, which is the language of a translation furnished for the purposes of:

☐ international search (under Rules 12.3 and 23.1(b))

☐ publication of the international application (under Rule 12.4(a))

☐ international preliminary examination (under Rules 55.2(a) and/or 55.3(a))

2. With regard to the **elements** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

☐ the international application as originally filed/furnished

☒ the description:
pages 1-22,27-29 and 33-46 as originally filed/furnished
pages* 23-26 and 30-32 received by this Authority on 06 September 2005 (06.09.2005)
pages* NONE received by this Authority on _____

☒ the claims:
pages 47-51 as originally filed/furnished
pages* NONE as amended (together with any statement) under Article 19
pages* NONE received by this Authority on _____
pages* NONE received by this Authority on _____

☒ the drawings:
pages 1/3-3/3 as originally filed/furnished
pages* NONE received by this Authority on _____
pages* NONE received by this Authority on _____

☐ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing.

3. ☐ The amendments have resulted in the cancellation of:

☐ the description, pages _____
☐ the claims, Nos. _____
☐ the drawings, sheets/figs _____
☐ the sequence listing (*specify*): _____
☐ any table(s) related to the sequence listing (*specify*): _____

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).

☐ the description, pages _____
☐ the claims, Nos. _____
☐ the drawings, sheets/figs _____
☐ the sequence listing (*specify*): _____
☐ any table(s) related to the sequence listing (*specify*): _____

* *If item 4 applies, some or all of those sheets may be marked "superseded."*

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/US05/03092

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims 5-8,25,26,28-30 and 32-34	YES
	Claims 1-4, 9-24, 27, 31	NO
Inventive Step (IS)	Claims 5-8, 25-26, 28-30, 32-34	YES
	Claims 1-4, 9-24, 27, 31	NO
Industrial Applicability (IA)	Claims 1-34	YES
	Claims NONE	NO

2. Citations and Explanations (Rule 70.7)

Claims 1-4, 9-24, 27 and 31 lack novelty under PCT Article 33(2) as being anticipated by GILBERTSON et al.

GILBERTSON et al disclose a demineralized bone matrix, and a stabilizing means (see column 11, lines 26-67). The implants may take the form of plates or screws (see column 12, lines 5-13). Carriers such as polysaccharides and collagen are found at column 13, lines 15-55. Bone morphogenic proteins are disclosed at column11, lines 39-44. The pH of the composition is also determined by its components and is an inherent property. The percent conductivity after one year is also an inherent property of the composition as set out by GILBERTSON et al. The instant claims are anticipated by GILBERTSON et al.

Claims 1-4, 9-24, 27 and 31 lack an inventive step under PCT Article 33(3) as being obvious over GILBERTSON et al.

GILBERTSON et al disclose a demineralized bone matrix, and a stabilizing means (see column 11, lines 26-67). The implants may take the form of plates or screws (see column 12, lines 5-13). Carriers such as polysaccharides and collagen are found at column 13, lines 15-55. Bone morphogenic proteins are disclosed at column11, lines 39-44. The pH of the implant may be adjusted by those of ordinary skill in the arts depending on where the implant is placed as well as the desired results of the pH adjustment. Those of ordinary skill would have expected a similar level of osteoinductivity at one year given that the same materials are set out by GILBERTSON et al, and this property is determined by the materials selected. The instant claims would have been obvious in view of the teachings of GILBERTSON et al.

Claims 5-8, 25, 26, 28-30, 32-34 meet the criteria set out in PCT Article 33(2)-(3), because the prior art does not teach or fairly suggest the instant demineralized bone matrix composition additionally containing water, hyaluronic acid, and protease inhibitors.

Claims 1-34 lack the criteria set out in PCT Article 33(4), and thus have industrial applicability because the subject matter claimed can be made or used in industry as a composition used for bone healing.

higher fatty acids such as lauric acid, myristic acid, palmitic acid, stearic acid, behenic acid, and arachidic acid.

[57] The addition of water substitutes to a composition also has the benefit of making the composition flowable and moldable.

[58] *Addition of stabilizing agents*. The incorporation of stabilizing agents into the inventive formulations is generally accomplished by suspending the molecule or molecules of interest in an appropriately compatible buffer as will be known to those skilled in the art. This buffer is then mixed with matrix in a relatively low liquid-to-solid volume ratio to form a slurry. Preferably, the pH of the buffer is approximately pH 7.4. In embodiments where the composition is acidified, the pH of the buffer is less than physiological pH is approximately the pH desired in the final DBM composition. In certain embodiments, the buffer with the stabilizing agent(s) is mixed with lyophilized matrix. The slurry may then be lyophilized and used to prepare the desired DBM formulations.

[59]

[60]

[61]

[62]

[63] In one embodiment, DBM particles are encapsulated with a thin layer of a water immiscible material. The encapsulated particles are then mixed with an aqueious solvent or carrier to produce a composition of the desired consistency. The coating excludes water from the DBM and serves to stabilize the activity. The water immiscible material is

preferably biodegradable, such as tyrosine polycarbonates, polyfumarates, tyrosine polyarylates, and poly-orthoesters such as polylactide, polygalactide, and co-polymers thereof. These polymers are biodegradable, and their properties can be modified by altering the chain length or degree of cross-linking of the polymer and/or the chemical structure of the monomers. Additionally, co-polymers can be prepared using combinations of resorbable polymers. Alternatively, a lipid or other lipophilic material is used. In many embodiments, the encapsulating material is dissolved in a volatile solvent such as an alcohol or chloroform. The solvent is then mixed with the DBM and lyophilized.

[[illegible]4] Enzyme inhibitors useful in the practice of the present invention may include, for example, acid protease inhibitors, serine protease inhibitors, metalloprotease inhibitors (*see* Whittaker *et al.* "Matrix Metalloproteinases and their Inhibitors-Current Status and Future Challenges" *Celltransmissions* 17(1):3-14; incorporated herein by reference), cysteine protease inhibitors, glyconase inhibitors, and glycosidase inhibitors. Specific protease inhibitors useful in the practice of the present invention

include, for example, aprotinin, 4-(2-aminoethyl)benzenesulfonyl fluoride (AEBSF), amastatin-HCl, alpha1-antichymotrypsin, antithrombin III, alpha1-antitrypsin, 4-aminophenylmethane sulfonyl-fluoride (APMSF), arphamenine A, arphamenine B, E-64, bestatin, CA-074, CA-074-Me, calpain inhibitor I, calpain inhibitor II, cathepsin inhibitor, chymostatin, diisopropylfluorophosphate (DFP), dipeptidylpeptidase IV inhibitor, diprotin A, E-64c, E-64d, E-64, ebelactone A, ebelactone B, EGTA, elastatinal, foroxymithine, hirudin, leuhistin, leupeptin, alpha2-macroglobulin, phenylmethylsulfonyl fluoride (PMSF), pepstatin A, phebestin, 1,10-phenanthroline, phosphoramidon, chymostatin, benzamidine HCl, antipain, epsilon-aminocaproic acid, N-ethylmaleimide, trypsin inhibitor, 1-chloro-3-tosylamido-7-amino-2-heptanone (TLCK), 1-chloro-3-tosylamido-4-phenyl-2-butanone (TPCK), trypsin inhibitor, sodium EDTA, and the TIMPs class of metalloproteinase inhibitors. Particularly useful ones are those stable under acidic conditions and effective at acidic conditions.

[65]

[66]

[67] *Covalent Modification of DBM.* The DBM may be covalently modified by the addition of polyethylene glycol or silylation.

agent so that the osteoinducing agent is available immediately upon implantation of the DBM.

[79] Osteoinducing agents include any agent that leads to or enhances the formation of bone. The osteoinducing agent may do this in any manner, for example, the agent may lead to the recruitment of cells responsible for bone formation, the agent may lead to the secretion of matrix which may subsequently undergo mineralization, the agent may lead to the decreased resorption of bone, *etc.* Particularly preferred osteoinducing agents include bone morphogenic proteins (BMPs), transforming growth factor (TGF-β), insulin-like growth factor (IGF-1), hormones including parathyroid hormone (PTH), and angiogenic factors such as VEGF. In one preferred embodiment (Example 12), the inducing agent is genetically engineered to comprise an amino acid sequence which promotes the binding of the inducing agent to the DBM or the carrier. Sebald *et al.* in PCT/EP00/00637, incorporated herein by reference, describe the production of exemplary engineered growth factors, suitable for use with DBM.

Formulation

[80] Improved osteogenic compositions of the present invention may be formulated for a particular use. The formulation may be used to alter the physical, biological, or chemical properties of a DBM preparation. A physician would readily be able to determine the formulation needed for a particular application taking into account such factors as the type of injury, the site of injury, the patient's health, the risk of infection, *etc.*

[81]

[82] Physical properties such as deformability and viscosity of the DBM may also be chosen depending on the particular clinical application. The particles of the improved DBM may be mixed with other materials and factors to improve other characteristics of the implant. For example, the improved DBM material may be mixed with other agents to improve wound healing. These agents may include drugs, proteins, peptides, polynucleotides, solvents, chemical compounds, biological molecules, *etc.*

[83] The particles of DBM (or inventive DBM material) may also be formed into various shapes and configurations. The particles can be formed into rods, strings, sheets, weaves, solids, cones, discs, fibers, wedges, coils, coiled coils, *etc.*

[84]

[85] In another embodiment of the invention, inventive DBM compositions having a pre-selected three-dimensional shape are prepared by repeated application of individual layers of DBM, for example by 3-D printing as described by Cima *et al.* U.S. Patents 5,490,962; and 5,518,680, each of which is incorporated herein by reference; and Sachs *et al.* U.S. Patent 5,807,437, incorporated herein by reference. Different layers may comprise individual stabilized DBM preparations, or alternatively may comprise DBM layers treated with stabilizing agents after deposition of multiple layers.

[86] In the process of preparing improved inventive DBM materials, the materials may be produced entirely aseptically or be sterilized to eliminate any infectious agents such as HIV, hepatitis B, or hepatitis C. The sterilization may be accomplished using any method or combination of methods, including one or more of antibiotics, irradiation, chemical sterilization (*e.g.*, ethylene oxide), or thermal sterilization. Other methods known in the art of preparing DBM such as defatting, sonication, and lyophilization may also be used in preparing the improved DBM. Since the biological activity of demineralized bone is known to be detrimentally affected by most terminal sterilization processes, care must be taken when sterilizing the inventive compositions. In preferred embodiments, the DBM compositions described herein will be prepared aseptically or sterilized as described in Example 6.

Applications

[87] Improved osteogenic compositions of the present invention may be used to promote the healing of bone injuries. The compositions may be used in any bone of the body on any type of injury. The improved DBM composition has been designed to